

The Application of a DNA-based Molecular Approach to Identify Sources of Fecal Contamination: The Search for Pathogens



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Bonita C. Johnson, Microbiologist

U.S. EPA, Region 4, Ecological Assessment Branch

Objectives

- Provide a brief introduction to the use of microbiological indicators to assess water quality.
- Explain the significance and purpose of using a DNA-based approach to identify probable sources of fecal contamination.
- Provide an overview of the PCR (Polymerase Chain Reaction and Agarose Gel Electrophoresis method and technique.

Why are we interested in these guys?

- Pathogens are known to cause harmful (even deadly) infectious diseases.
- Of the designated uses listed in section 303c of the Clean Water Act, protection from pathogenic contamination is most important for waters designated for recreation, public water supplies, aquifer protection; and protection and propagation of fish, shellfish, and wildlife.

Have committed violations associated
with the following:

- Safe Drinking Water Act
- Total Coliform Rule
- Surface Water Treatment Rule
- Clean Water Act
- Beach Act 2000
- Total Maximum Daily Load (TMDL)
- Ground Water Rule
- Confined Animal Feed Operations (CAFOs)
- Water Quality Standards

Known Hang-outs

Primary Sources:

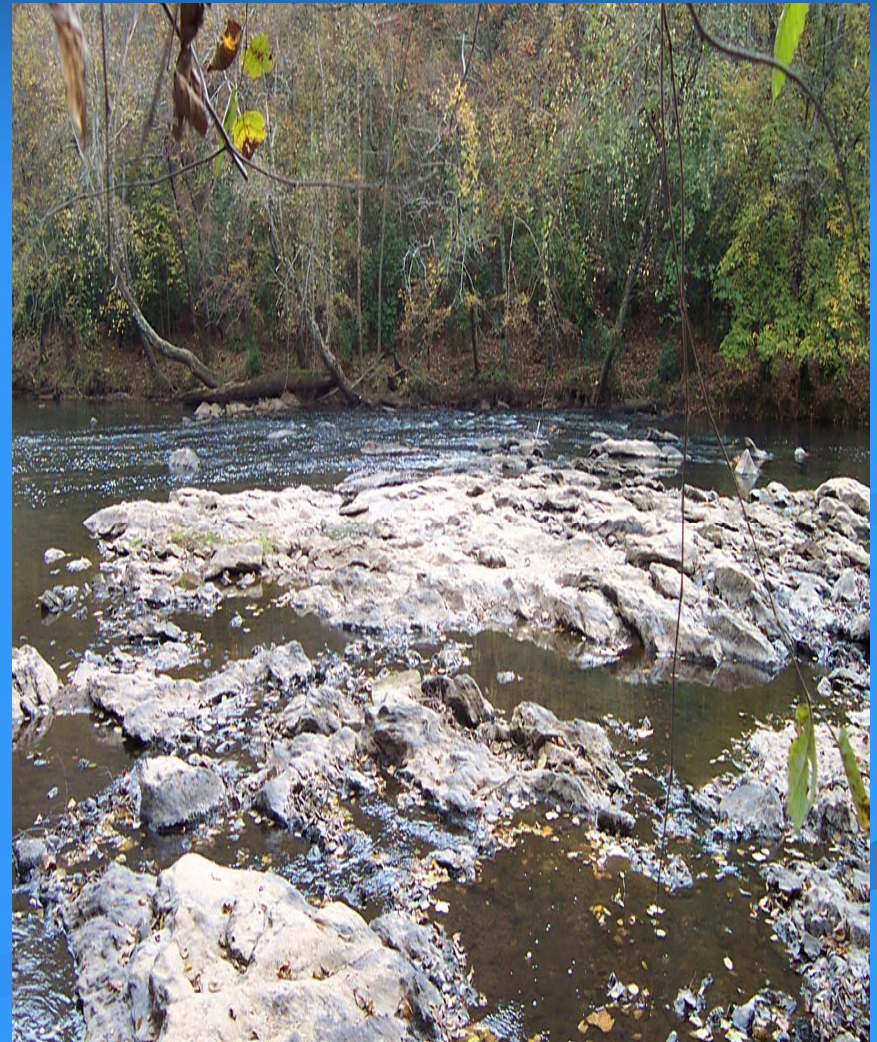
Agricultural operations -
livestock, manure

Septic tank systems

Drinking Water
Systems

Wastewater Treatment
Systems

Recreational waters-
streams, lakes, ponds



Rap Sheet: Outbreaks of Disease

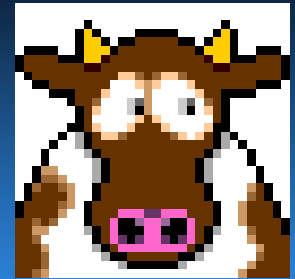
- 1909 England
- 1921 Connecticut
- 1932 New York
- 1942 California
- 1947 England
- 1958 Australia
- 1973 Vermont
- 1974 France
- 1974 South Carolina
- 1976 Iowa
- 1982 Michigan

Modus Operandi (MO)

Usually enter surface and groundwater by means of:

- Flood events – storm water run-off.
- Abandoned wells - provide a direct channel for contaminants to pollute the aquifer below.
- Poorly constructed wells - allow for contamination from septic tanks placed too close.
- Spills - material spilled or dumped near a well can leach into the aquifer and contaminate the groundwater drawn from a well.

Last known major crime: Surface Water Contamination Walkerton, Ontario



- Rural farm community
- The well water was contaminated by surface water carrying livestock waste immediately after heavy rain storms.
- The drinking water was insufficiently chlorinated to kill *E. coli* from the manure, causing the outbreak.
- A virulent strain of intestinal bacteria left 7 dead and thousands ill. Phenotypic and molecular typing methods confirmed the presence of a single *E. coli* type - O157:H7 and a variety of *Campylobacter* spp. strains, which were also found in cattle from nearby farms.

How do we search for pathogens?

By using indicator organisms:

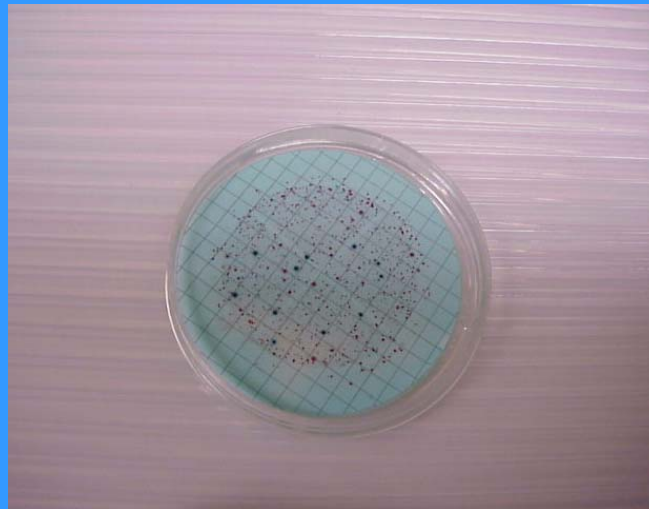
- Relatively easy to isolate.
- Survive longer than most disease-producing organisms.
- Are almost always present in water containing enteric pathogens.



The Usual Suspects

- Total Coliform- primarily used to assess potable water acceptability.

Last known place of residence: naturally occurs and can be found in plants and soils.



>1000 cfu/100mL total coliform and 33 cfu/100mL *E. coli*

Known Associates

- Fecal Coliform- primarily used to assess wastewater and other non-potable water quality.

Last known place of residence: gastrointestinal tract of humans and warm-blooded animals.

- *E. coli*- recommended to assess quality of fresh water.

Last known place of residence: gastrointestinal tract of humans and warm-blooded animals.

- Enterococci- recommended to assess quality of marine water.

Last known place of residence: gastrointestinal tract of humans and warm-blooded animals.

Water Quality Criteria

- fecal coliform
200 cfu/100 mL
- *E. coli*
126 cfu/100 mL
- enterococci
33 cfu/100 mL



fecal coliform bacteria

How do we address this issue?

By exploring the use of innovative approaches such as Microbial Source Tracking.

- Microbial Source Tracking (MST) is a relatively novel approach that is considered to be the best tool available for identifying sources of fecal pollution in water.
- It is based on the assumption that specific species and strains of bacteria are associated with specific hosts.

Who are you?



Bacterial Source Tracking

Types of Methods

- Molecular (genotype) are all referred to as "DNA fingerprinting" and are based on the unique genetic makeup of different strains, or subspecies, of fecal bacteria.
- Biochemical (phenotype) methods are based on an effect of an organism's genes that actively produce a biochemical substance. Chemical methods are based on finding chemical compounds that are associated with human wastewater.

Bacterial Source Tracking

- Develop a library or database of isolates taken from known sources (e.g. human, cow, deer, etc.).
- The size of the library will be partly determined by the number of potential major sources of fecal pollution in the target area.

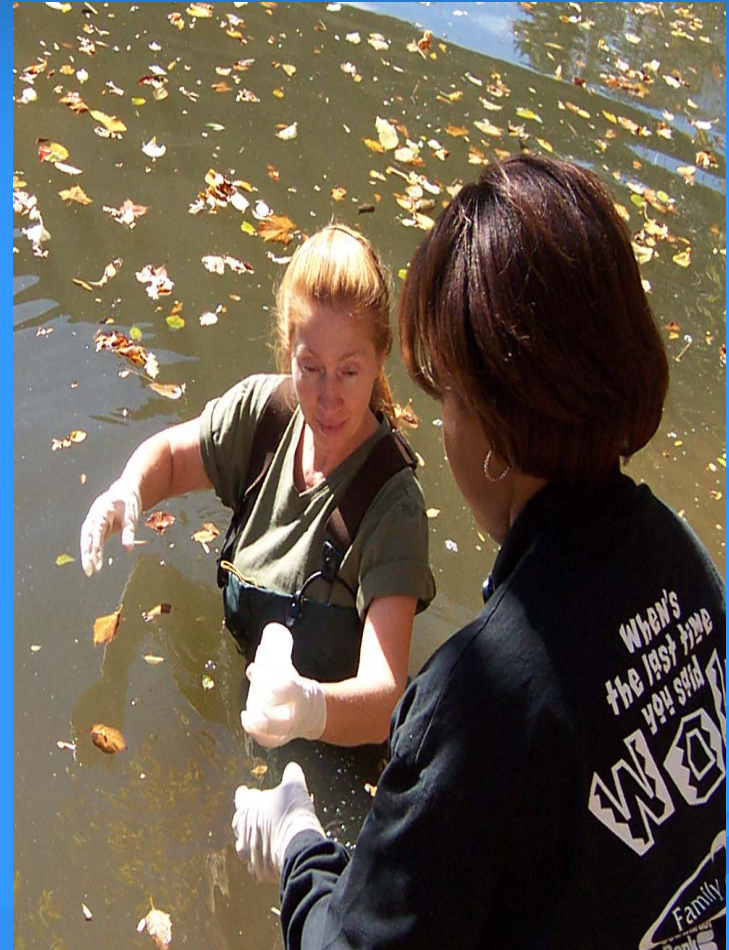
Which indicator will we go after?

E. coli or enterococci

- persists longer in the environment than *E. coli*
- can survive in adverse conditions (salt water conditions)
- species are more source specific than *E. coli*
- found in 80-90% of clinical isolates taken from humans with infections

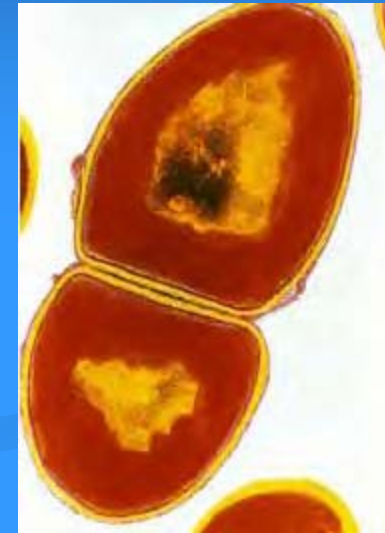
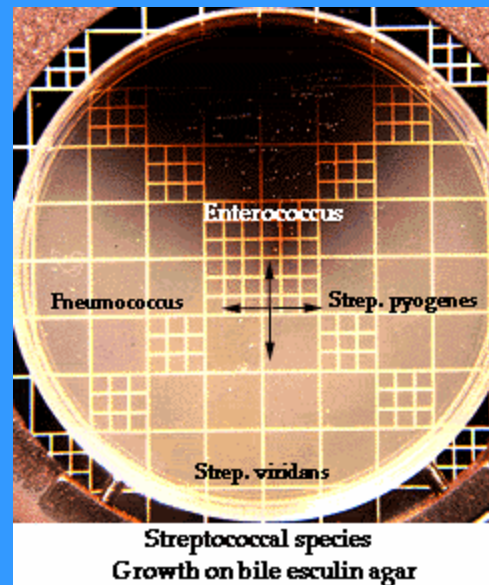
Sampling Plan

- Collected surface water and cow manure samples at Chandler Farm in Athens, GA.
- Chandler Farm is located in the Broad River Watershed.
- The watershed is impaired due to excessive nutrients and pathogens.



The Capture and Isolation of Enterococci

EPA Method 1600-mEI agar



On the Road to PCR

- Verify enterococci
- Subculture isolates for purity
- Inoculate the template or vials with sample
- Prepare master mix-unique and specific primers



PCR Cycling Reactions

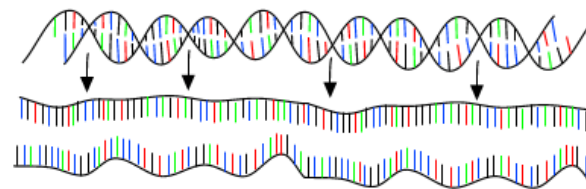
- Inoculate the template with master mix
- Load the template into the Thermal Cycler
- A typical run takes 2 hours



PCR Cycling Steps

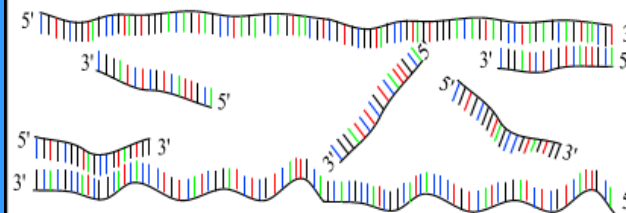
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

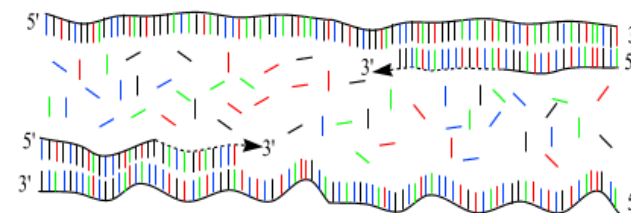
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse
primers !!!

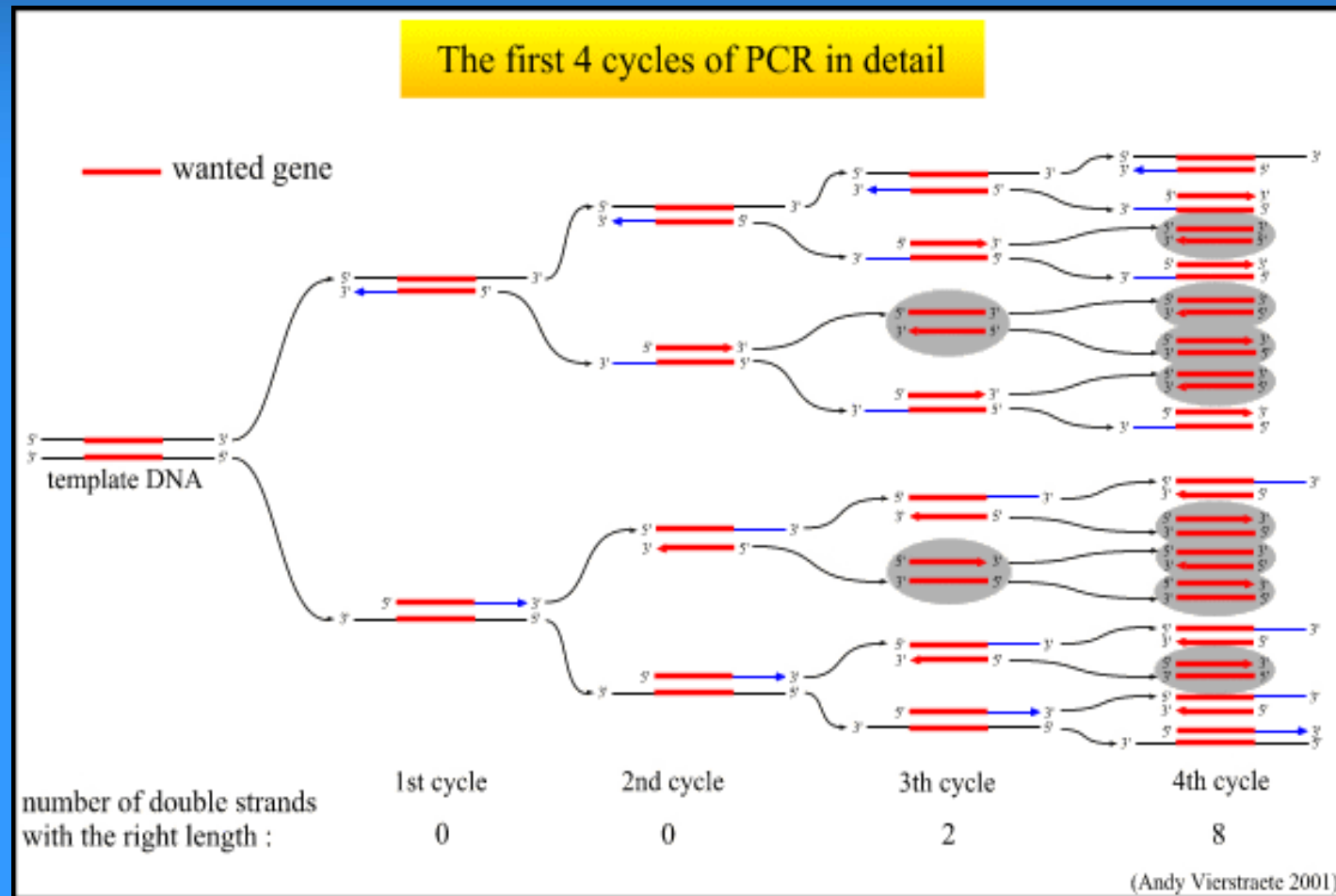


Step 3 : extension

2 minutes 72 °C
only dNTP's

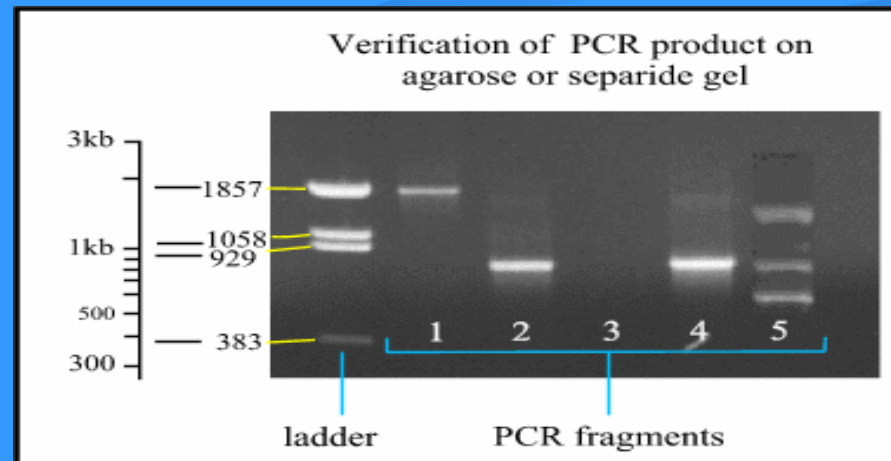
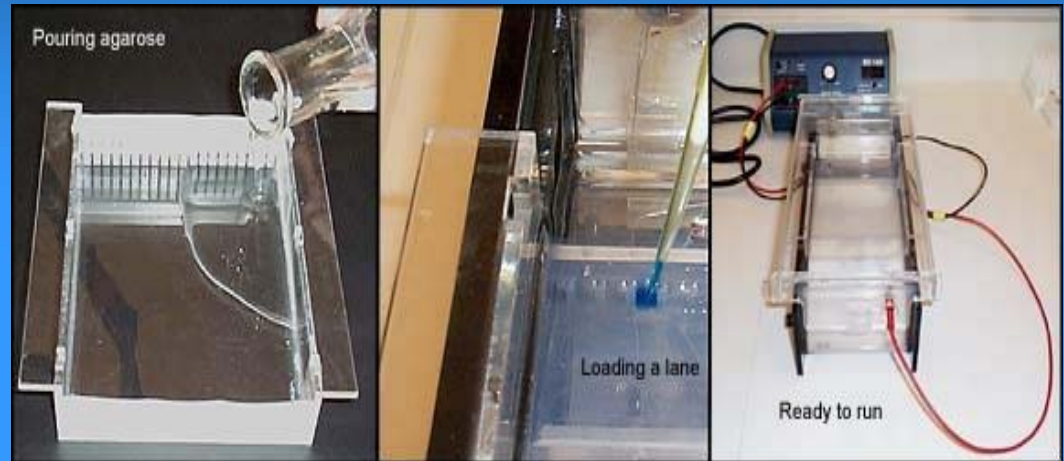
(Andy Vierstraete 1999)

Polymerase Chain Reaction



Agarose Gel Electrophoresis

- An electrical voltage is applied to the PCR product to cause the DNA fragments to migrate.
- The distance that the DNA fragments migrate is related to their molecular weights and number of base pairs.



Visualization of DNA Product



Lane 1-DNA marker, 2-no DNA, 3-lactococcus, 4-11-Enterococcus-genus and faecalis-species markers; enterococci isolated from streams in the Broad River Watershed, Athens, GA. Sept. 2003, Lane 12-DNA marker.

Enterococci isolates identified thus far include:

Water

E. faecalis

E. faecium

E. columbae

E. avium

E. casseliflavus

Manure

E. faecalis

E. faecium

E. columbae

E. avium

E. hirae

E. raffinosis

Ongoing Related Work

- The staff of Region 4 will continue to determine the species of enterococci isolates obtained from the Broad River Watershed, and will contribute to ongoing work with ORD/NERL and USDA/ARRU-Athens.
- Region 4 will maintain in-house PCR/Electrophoresis and Microbial Source Tracking capabilities.
- Region 4 is supporting an Inter-RARE Project to develop a national guidance document for using Microbial Source Tracking.

Ongoing Related Work

- The Homeland Security, Biological Methods Review Subteam, with contractor support, will publish a document which summarizes methods recommended for detecting biological agents. PCR, with culture techniques, is the single, most prevalent method for detecting agents of concern.

Resource Documents

- Ambient Water Quality for Bacteria – 1986
- National Recommended Water Quality Criteria-Correction – 1999
- Implementation Guidance for Ambient Water Quality Criteria for Bacteria-2002
- The above documents can be found at <http://www.epa.gov/waterscience/humanhealth/microbial/microbial.html>

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Bonita C. Johnson, Microbiologist
US EPA, Region 4
Science and Ecosystem Support Division
Ecological Assessment Branch
johnson.bonita@epa.gov
706-355-8725

